

WHAT IS CLAIMED IS:

- 1 1. A conditional replication shuttle vector comprising:  
2 (a) an R6K $\gamma$  origin of replication; and  
3 (b) a nucleic acid encoding a recombination protein.
- 1 2. The conditional replication shuttle vector of Claim 1 wherein the recombination  
2 protein is recA
- 1 3. The conditional replication shuttle vector of Claim 1 further comprising a  
2 nucleic acid encoding a marker protein.
- 1 4. The conditional replication shuttle vector of Claim 3 wherein the nucleic acid  
2 encoding the marker protein is IRES-EGFP.
- 1 5. The conditional replication shuttle vector of Claim 3 further comprising a  
2 second marker protein.
- 1 6. The conditional replication shuttle vector of Claim 5 wherein the nucleic acid  
2 encoding the second marker protein is taulacZ.
- 1 7. The conditional replication shuttle vector of Claim 1 further comprising a gene  
2 that can be counter-selected against.
- 1 8. The conditional replication shuttle vector of Claim 7 wherein the gene that can  
2 be counter-selected against is SacB.
- 1 9. The conditional replication shuttle vector of Claim 7 wherein the gene that can  
2 be counter-selected against confers tetracycline resistance.

7 wherein the host cell comprises an IOBCV which contains the particular  
8 nucleotide sequence; wherein the conditional replication shuttle vector encodes a  
9 recombination protein that is transiently expressed by the host cell; wherein the  
10 conditional replication shuttle vector contains a nucleic acid that selectively integrates  
11 into the particular nucleotide sequence when the recombination protein is expressed;  
12 and wherein neither the IOBCV alone, nor the IOBCV in combination with the host  
13 cell can independently support homologous recombination.

1 16. A method of selectively modifying a particular nucleotide sequence of an  
2 independent origin based cloning vector (IOBCV) that is contained in a recombination  
3 deficient host cell comprising:

4 (a) introducing a conditional replication shuttle vector into a recombination  
5 deficient host cell; wherein the host cell comprises an IOBCV that comprises a gene of  
6 interest which contains the particular nucleotide sequence; wherein the conditional  
7 replication shuttle vector encodes a recombination protein that is expressed by the host  
8 cell and permits homologous recombination to occur in the host cell; wherein the  
9 conditional replication shuttle vector contains a nucleic acid that selectively integrates  
10 into the particular nucleotide sequence when the recombination protein is expressed  
11 forming a co-integrate; wherein the nucleic acid that selectively integrates into the  
12 particular nucleotide sequence and the nucleic acid encoding the recombination protein  
13 are positioned on the conditional replication shuttle vector such that upon resolution of  
14 the co-integrate, the nucleic acid encoding the recombination protein remains with the  
15 conditional replication shuttle vector; and wherein neither the IOBCV alone, nor the  
16 IOBCV in combination with the host cell can independently support homologous  
17 recombination; and

18 (b) growing the host cell under conditions in which the conditional  
19 replication shuttle vector cannot replicate, therein diluting out the conditional  
20 replication shuttle vector encoding the recombination protein, and thereby preventing  
21 further recombination events in the recombination deficient cells.

1 17. The method of Claim 16 wherein the conditional replication shuttle vector  
2 further comprises a nucleic acid that encodes a marker protein or peptide and wherein  
3 the nucleic acid that selectively integrates into the particular nucleotide sequence and  
4 the nucleic acid encoding the marker protein or peptide are positioned on the  
5 conditional replication shuttle vector such that upon resolution of the co-integrate, the  
6 nucleic acid encoding the marker protein or peptide is inserted into or adjacent to the  
7 particular nucleotide sequence.

SUB B2  
COT

09619364-071900

SUB B3) 1 18. The method of Claim 16 wherein the conditional replication shuttle vector  
2 cannot replicate in the host cell because the conditional replication shuttle vector  
3 requires a particular protein for replication and neither the host cell nor the IOBCV  
4 encode the particular protein.

1 19. The method of Claim 16 wherein the IOBCV is a BBPAC.

SUB B4) 1 20. The method of Claim 19 wherein the BBPAC is a BAC.

1 21. The method of Claim 20 wherein the conditional replication shuttle vector  
2 cannot replicate in the host cell because the conditional replication shuttle vector  
3 comprises a R6K $\gamma$  origin of replication and neither the host cell nor the BAC encode  
4 pir.

1 22. The method of Claim 21 wherein the conditional replication shuttle vector  
2 further comprises a first frt site that is positioned on one side of the nucleic acid that  
3 selectively integrates into the particular nucleotide sequence, and a second frt site that  
4 is positioned on the other side of the nucleic acid that selectively integrates into the  
5 particular nucleotide sequence and wherein the resolution of the co-integrate is  
6 performed by adding flip recombinase to the host cell.

1 23. The method of Claim 21 wherein the conditional replication shuttle vector  
2 further comprises a nucleic acid encoding a marker protein or peptide that is positioned  
3 in between the two frt sites and is also adjacent to the nucleic acid that selectively  
4 integrates into the particular nucleotide sequence such that after the resolution, the  
5 marker protein or peptide is contained by the BAC.

1 24. The method of Claim 22 wherein flip recombinase is added to the host cell by  
2 introducing a plasmid that encodes flip recombinase to the host cell.

1 25. The method of Claim 24 wherein the plasmid contains a conditional origin of  
2 replication.

00572043667960

[illegible]

- 1 26. The method of Claim 21 wherein the conditional replication shuttle vector  
2 further comprises two homologous nucleotide sequences that are homologous to each  
3 other but are not homologous to the BAC; wherein the two homologous nucleotide  
4 sequences are positioned on the conditional replication shuttle vector to be on opposite  
5 sides of the nucleic acid that selectively integrates into the particular nucleotide  
6 sequence; and wherein the resolution of the co-integrate is performed by a  
7 recombination event between the two homologous nucleotide sequences.
- 1 27. The method of Claim 26 wherein the two homologous nucleotide sequences are  
2 IRESEGFP.
- 1 28. The method of Claim 21 wherein the recombination deficient host cell cannot  
2 independently support homologous recombination because the host cell is RecA<sup>-</sup>.
- 1 29. The method of Claim 21 further comprising adding a counterselection agent  
2 after the resolution of the co-integrate to remove host cells that comprise the  
3 conditional replication shuttle vector; wherein the conditional replication shuttle vector  
4 further comprises a counterselection gene that is positioned on the conditional  
5 replication shuttle vector such that upon resolution of the co-integrate the  
6 counterselection gene remains with the conditional replication shuttle vector.
- 1 30. The method of Claim 29 wherein the counterselection agent is sucrose and the  
2 counterselection gene is SacB.
- 1 31. The method of Claim 30 wherein the recombination deficient host cell cannot  
2 independently support homologous recombination because the host cell is RecA<sup>-</sup>.
- 1 32. The method of Claim 31 wherein the recombination protein is selected from the  
2 group consisting of recA, the rec E and rec T protein pair, the Lambda *beta* protein,  
3 and the *Arabidopsis thaliana* DRT100 gene product.

1 33. An IOBCV that comprises a particular nucleotide sequence that has been  
2 modified by the method of Claim 16.

1 34. A BAC that comprises a particular nucleotide sequence that has been modified  
2 by the method of Claim 21.

1 35. A BAC that comprises a particular nucleotide sequence that has been modified  
2 by the method of Claim 26.

1 36. A method of producing a non-human transgenic animal comprising:  
2 (a) introducing a Bacterial or Bacteriophage-Derived Artificial  
3 Chromosome (BBPAC) into a eukaryotic cell; and  
4 (b) placing the eukaryotic cell into a recipient animal, wherein the  
5 eukaryotic cell develops into the non-human transgenic animal;  
6 wherein the BBPAC has been modified through homologous recombination in  
7 a RecA<sup>+</sup> bacterial host cell that has been induced to support homologous recombination  
8 by the transient expression of a recombination protein; and wherein the eukaryotic cell  
9 is selected from the group consisting of a fertilized animal zygote and an embryonic  
10 stem cell.

1 37. The method of Claim 36 wherein said eukaryotic cell is a fertilized animal  
2 zygote and said introducing is performed by pronuclear injecting the BBPAC into the  
3 fertilized animal zygote.

1 38. The method of Claim 37 wherein the BBPAC is a Bacterial Artificial  
2 Chromosome (BAC); the animal is a mouse; and the fertilized animal zygote is a  
3 C57BL/6 mouse zygote.

1 39. The method of Claim 38 wherein said eukaryotic cell is a mouse embryonic  
2 stem (ES) cell and said introducing is performed by transfecting the mouse ES cell.

1 40. The method of Claim 39 wherein the BBPAC is a Bacterial Artificial  
2 Chromosome (BAC).

- 1 41. The method of Claim 40 wherein the animal is a mammal.
- 1 42. The method of Claim 41 wherein the mammal is a mouse.
- 1 43. A method of producing a non-human transgenic animal comprising:  
2 (a) introducing the BAC of Claim 34 into a eukaryotic cell; and  
3 (b) placing the eukaryotic cell into a recipient animal, wherein the  
4 eukaryotic cell develops into the non-human transgenic animal.
- 1 44. A method of producing a non-human transgenic animal comprising:  
2 (a) introducing the BAC of Claim 35 into a eukaryotic cell; and  
3 (b) placing the eukaryotic cell into a recipient animal, wherein the  
4 eukaryotic cell develops into the non-human transgenic animal.
- 1 45. A non-human transgenic animal obtained by the method of Claim 44.
- 1 46. A non-human transgenic animal obtained by the method of Claim 43.
- 1 47. A non-human transgenic animal obtained by the method of Claim 36.

ADD B1)